

FIG. 1

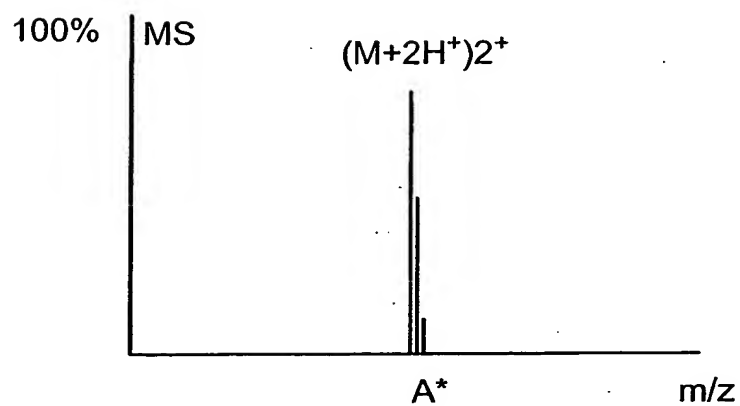


FIG. 2

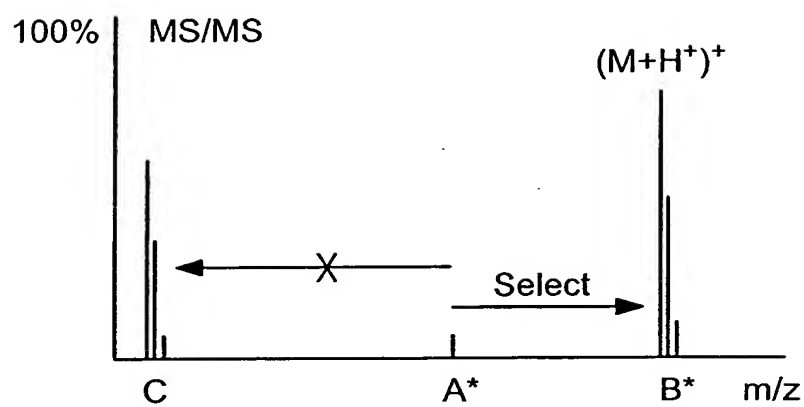


FIG. 3A

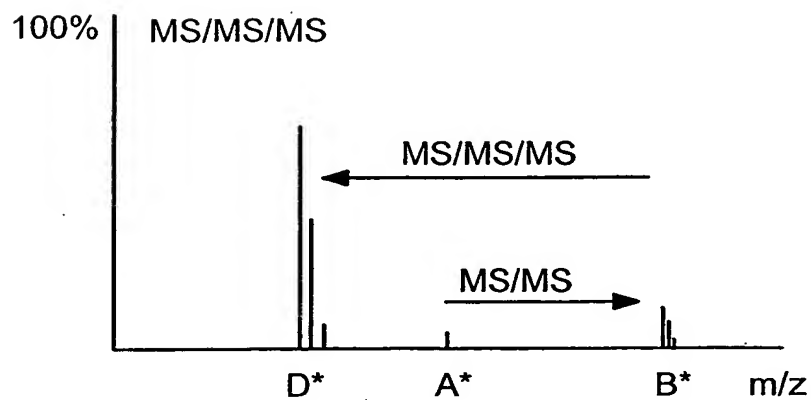


FIG. 3B

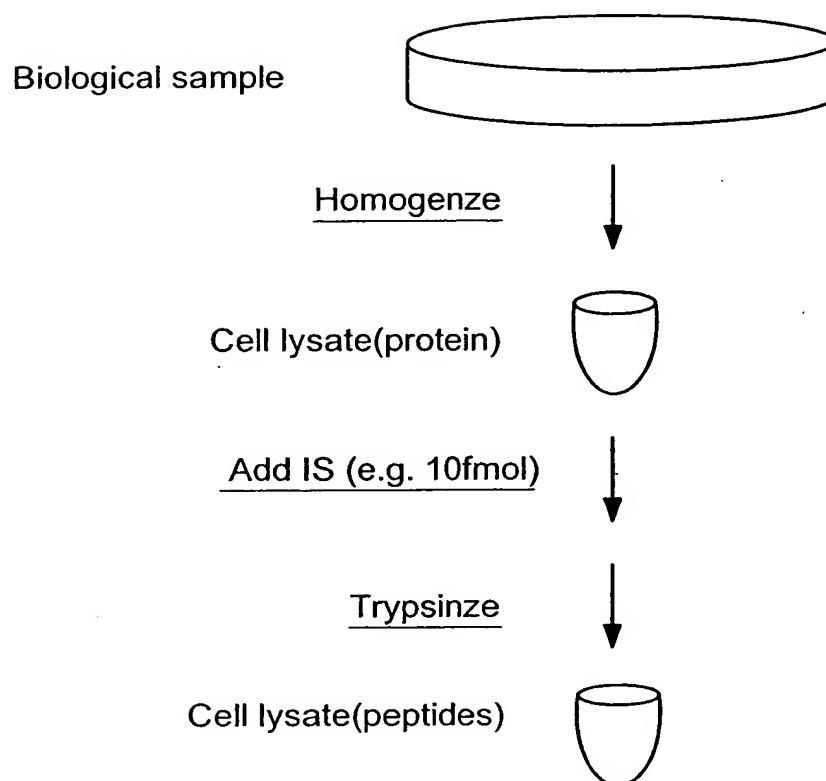


FIG. 4A

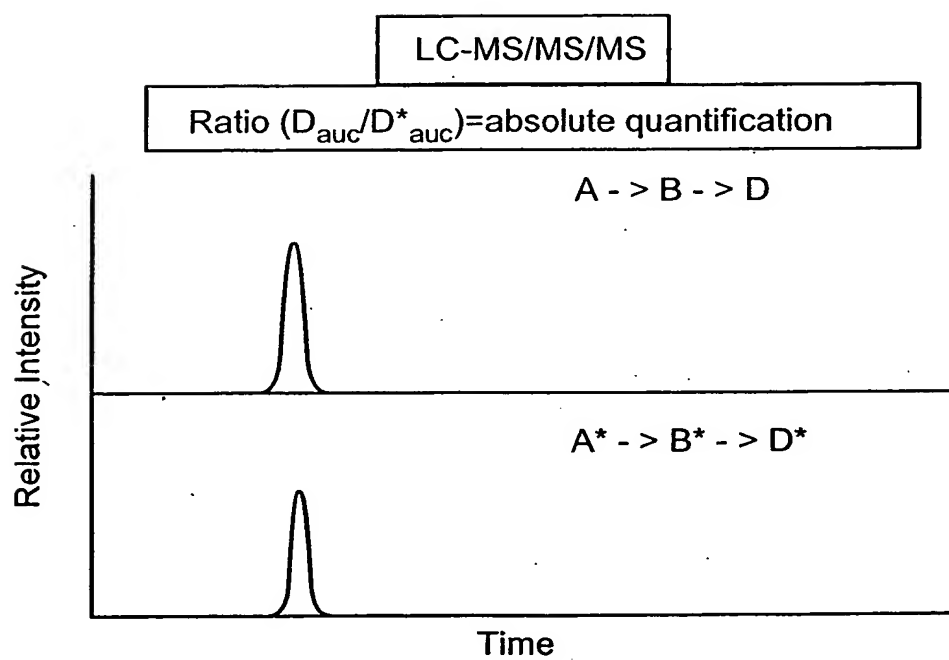
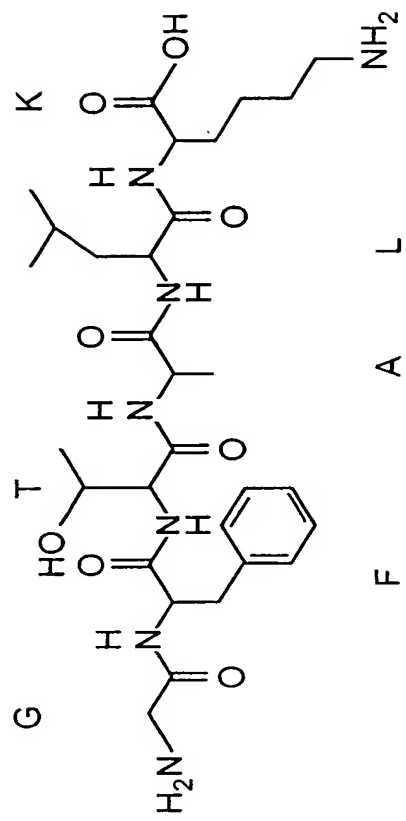


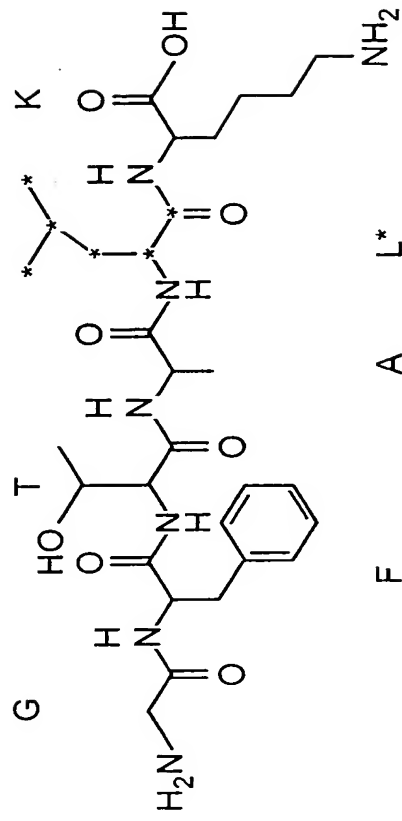
FIG. 4B



Native tryptic peptide

GFTALK

MW: 635.364



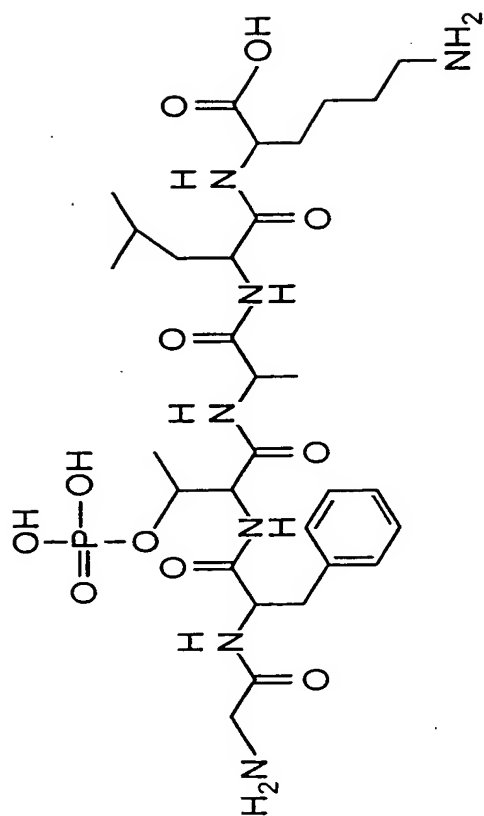
AQUA Internal Standard

GFTAL*K

MW: 641.364

* = stable isotope (e.g. ¹³C)

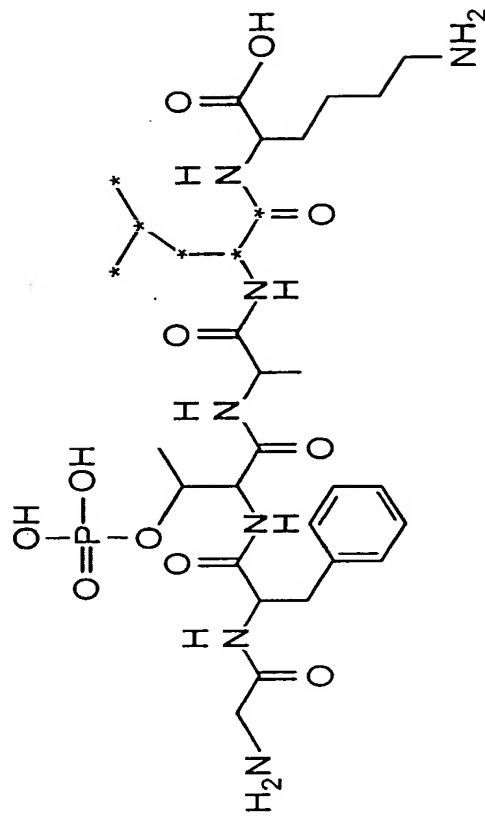
FIG. 5A



Native phosphopeptide

GF(pT)ALK

MW: 715.754

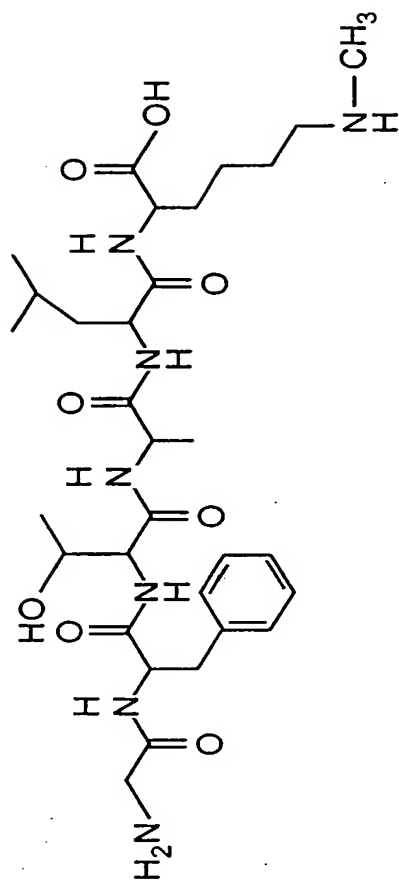
AQUA phosphopeptide
Internal Standard

GF(pT)AL*K

MW: 721.75

* = stable isotope (e.g. ^{13}C)

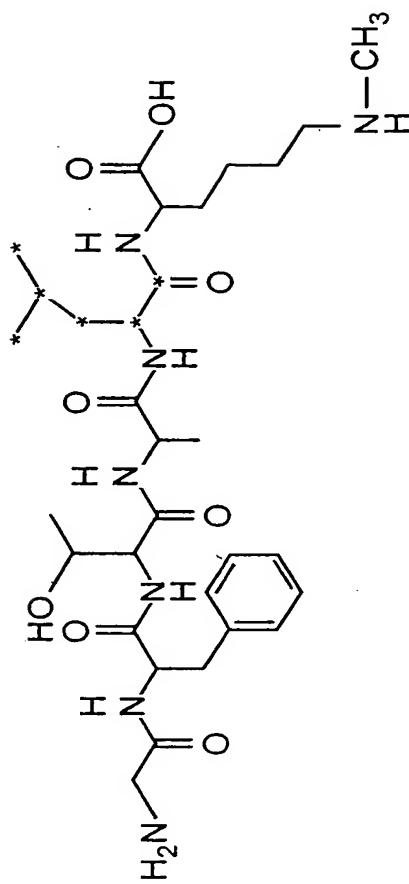
FIG. 5B



Native methylated peptide

GFTAL(mK)

MW: 649.79



AQUA methylated peptide
Internal Standard

GFTAL*(mK)

MW: 655.79

* = stable isotope (e.g. ^{13}C)

FIG. 5C

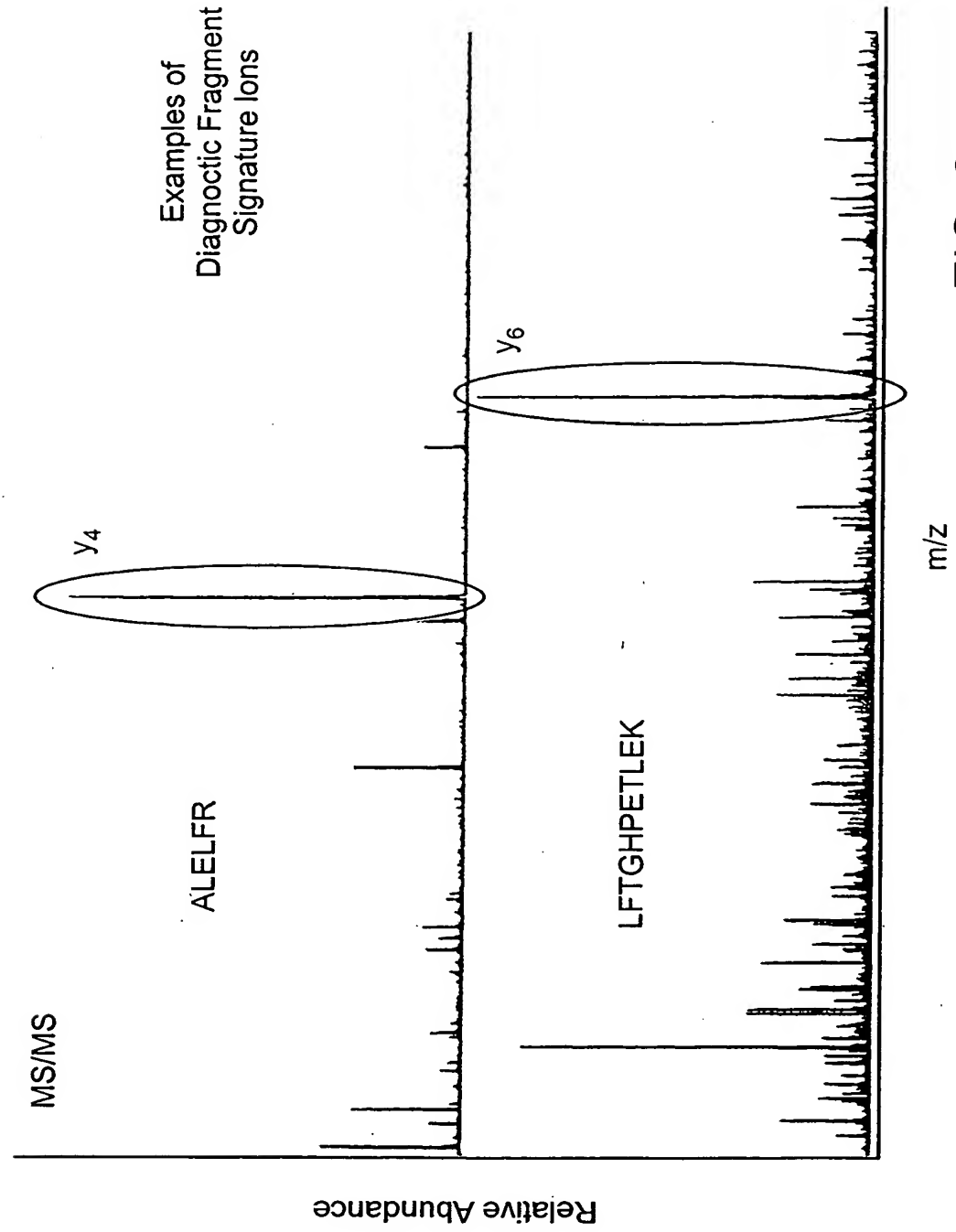


FIG. 6

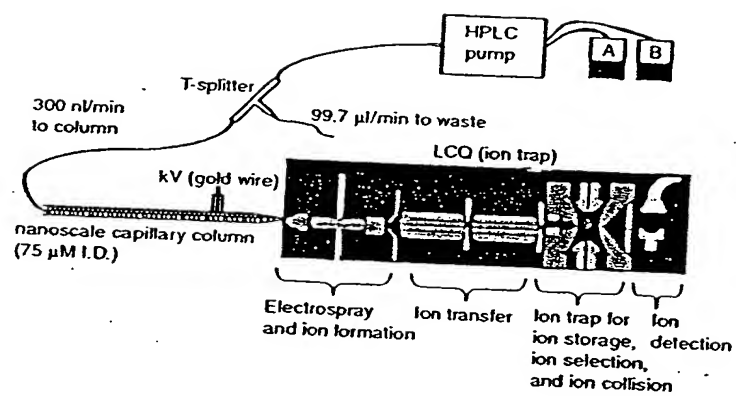
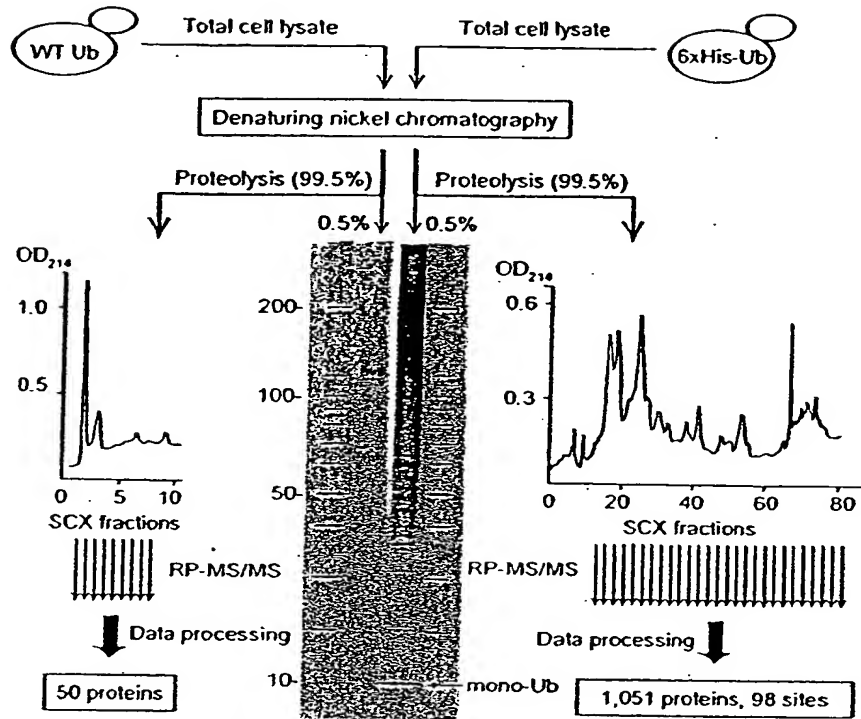


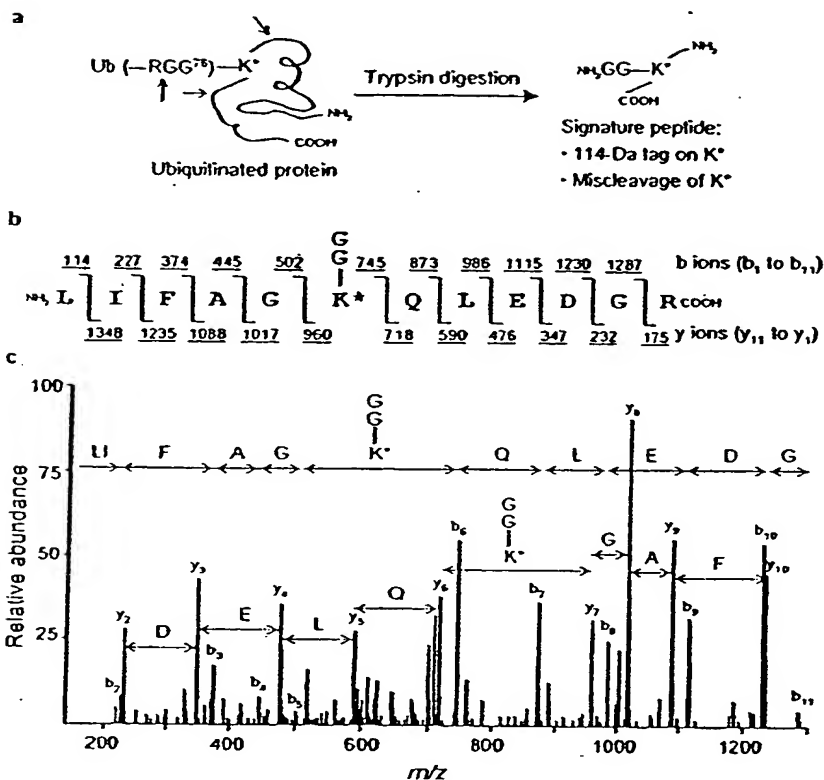
Figure 7

Figure 8



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Figures 9A-9C



Figures 10A-10C

a

Name	#sites	Sequence
Ubiquitin	5	MPFI TSRPVA KNSHSELSET ELNOSKQPF QSPDTOLGS AQQRSSSTI RQALSSELGG
ECM21	5	AMHSPAVLN NITKCGNNG N RSSNTDAQ LLGQKQKQF PPNNRRHSTT AI QCSI SDSA
SAM2	4	TTTPRSSTD THRTSQRLS YDQEPRI SGG RYSQ EEDST YLIFDDHNS SAVVSSILSS
YHR097C	3	TSLTRLANSK KTFEQLI EY LTARGLLPK TVLSNEYLI SI STSGESVF LPTI SSNICE
HXT7	3	YLSRLNLND GTDDAEADF MGG DQEQN TPLATTAA TESQSI NEN RUTLLRNN
GNP1	3	GDHPGSGEL NTRSVI DSS MYSYI AVI V SVGGTRFTD MQLCLSRVK VFWNTGVPPT
YIL041W	2	KTFNEEFYNA ASKOWUNDE NDLFVPLSI SPDDQM ENN SNDRQRLFK N PTEERLYL
YHL010C	2	DKTKKASLL NW DYNKTHL YQDQVFLV PVVFSNH PE TI YLPSARVS YRLRLATKA
LSB1	2	NRKGFYRQS NSPQPI VSPD SSSSLSSSTS SLQLTETESA QAHRI SNTL FSKVQNHUM
ZE01	2	SSHQLQNEES GEEDFAEYE IXM KTFPEY AVSTANPLY IIRVWISLS YEI SFAQCYV
PHO84	2	SLNKEVPI KI KLAPI QONVC VKR HYSI TE RVTFYSKQIE YEDQITLVA KQFNYLYLD
URA3	2	FASKRRKERS VSLFEI RTKE KGTALREED VENSFNQLE SYSPFDCEED SNGPKERLG
GDH1	2	I TEPI I ETK LKPKYEDLD KRTAQ I PPY Q DAYTSI PN PEHAVANGPS HRRPSMGE
ERG5	2	SGKCKSKIE DNEKPYDPK FIKZII ISN QLVKTHRL NTPKRLYLD SLHFSNVYR
ERG3	2	HQLEIMRIS KPDPEQSKL RHYEVL DTP I FLVSEQQS QNELPTYDM ATMECKNQV
YGR268C	2	PLSMGDFTG NTPPPPTFE EAI SVFASPI VSPMGPN M ASYDILLSI QQLNLSRTTS
YMR295C	2	VSGPSGSED AGVNNNRNS I SNANMNS I SNAFVSN SOQGVARARA TSVNDRSRFN
		NLDKLLSTPS PVRSHNSP TRLSQNGT VRI PNATTEN SKIKQNEFTK KGYTLANVXD
		DEEQEQ VSS SSADSLSSNG NEPPRYDEI V PLNDEE

b

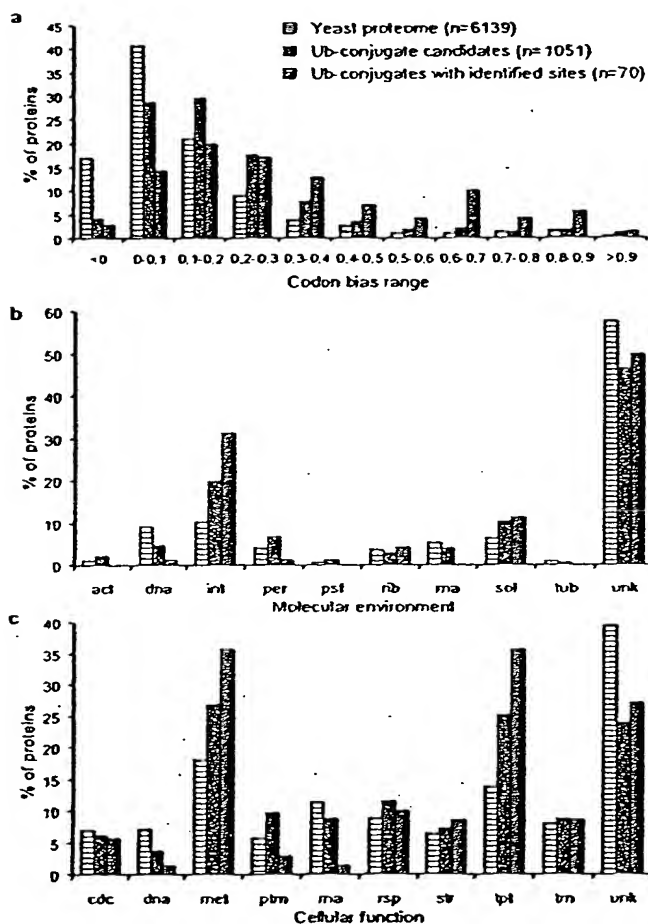
Site in Ub (K)	Signature peptide	SCX fraction	Abundance
48	LIFAGK*QLEDGR	48-56 (9)	high
63	TLSDYNQK*ESTLHLVLR	65-72 (8)	high
11	TLTGK*TITLEVESSDTIDNVK	38-42 (5)	medium
27	TITLEVESSDTIDNVK*SK	41 (1)	low
6	LISEEDLGMQIFVK*TLTGK	38 (1)	low

c

Name	Phosphopeptide sequence	Description of function
ACC1	AVS*YSDLSYVANSQSSPLR	Acetyl-CoA carboxylase
CCC1	GGGGTSELGGSEST*PLLK	Protein potentially in calcium regulation
CH01	DENDGYAS*DEVGGLSR	Phosphatidylserine synthase
CHS1	DDEYDDLNT*IDK	Chitin synthase I
CHS3	NPSTLLPTS*SMFWNK	Chitin synthase III
ECM21	NEES*GEEDFAEYPIK	Protein possibly in cell wall biosynthesis
	HALSS*LLGGANVHSPAVLNNTTK	
	RPS*VIGFLSGHK	
	S*HNSS*PTINGLSQANGTVR	
GCD6	EEIDS*EFEDDFEK	Translation initiation factor eIF2B
HSP30	ASGETAHEPEPEAEQAVEDT*A	Heat shock protein located in cell membrane
LYP1	LQVVSHE*DNEDDEEAHYEDK	High affinity lysine-specific permease
MET4	KYS*DNEDDEYDDADLHGFEK	Transcriptional activator
MYO3	RGS*YYHVPLNPVQATAVR	Myosin type I
PHO84	IHDTS*DEDMAINGLER	Inorganic phosphate/H ⁺ symporter
	NNDIESS*PSQLQHEA	
RAD16	SVNYNELS*DDDTAVK	Nucleotide excision repair protein
Ubiquitin	TLSDYNQK	Protein for posttranslational modification
YDR119W	IEEINENS*PLLSAPSK	Member of major facilitator superfamily (MFS)
YDR348C	TNS*FDMPOLNTR	Protein of unknown function
YHR097C	ETVDDDS*ETLNQLQDR	Protein of unknown function
	LPSYEEAAGT*PK	
YOR042W	KNPDEDEFINS*DDDM	Protein of unknown function
	SSGIDEDEVVT*PAEDAKEEEEHPPLPAR	
	EOHHEDS*EEEDSWOFEK	
YPL019C	HYADLEDHES*S*DEEGTALPK	Vacuolar polyphosphate accumulation

a, Proteins identified containing multiple ubiquitination sites. **b**, Five lysine residues in ubiquitin were found to be sites for (poly)ubiquitination. The number in parenthesis indicates the total fractions in which the signature peptide was detected. **c**, List of phosphorylated peptides from the Ub-conjugates candidates. Phosphorylated residues are indicated by asterisk. Three peptides contained multiple (two) phosphorylation sites. There were 19 proteins identified from 26 phosphopeptides with 29 total phosphorylation sites. Of special interest is the phosphorylation of S⁵⁷ in Ub itself. The sequence of ECM21p is also shown with 5 ubiquitination and 5 phosphorylation sites highlighted. Sixty five other unmodified peptides were also identified from this protein (not shown for clarity).

FIGURES 11A-11C



Comparison of protein expression, environment, and function among the yeast proteome, 1,051 candidate Ub-conjugates, and 70 proteins with detected Ub sites. **a**, Codon bias value (an indicator of protein expression levels with a value < 0.1 indicating likely low-copy-number proteins). **b**, protein molecular environment. act: actin-associated; dna: DNA-associated; int: integral membrane; per: peripheral membrane; psf: protein synthesis factor; rib: ribosome-associated; sol: soluble; tub: tubulin-associated; ma: RNA-associated; unk: unknown. **c**, cellular function. cdc: cell division control; dna: DNA recombination, replication and repair; met: metabolism; ptm: protein posttranslational modification and degradation; ma: RNA transcription, processing and turnover; rsp: responses to environment; str: cellular structure; tpt: transport of proteins and small molecules; trn: protein translation and assembly; unk: others and unknown.

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